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ANNUAL REPORT TO THE U.S. ARMY MEDICAL

RESEARCH AND DEVELOPMENT COMMAND, DEPARTMENT OF THE ARMY

Title of study/report:	"Calciphylaxis in wound healing;" number of pages: 4
Period covered by report:	September 1st, 1962 -- August 31st, 1963.
Responsible investigator:	Hans Selye, Professor and Director, Institute of Experimental Medicine and Surgery.
Name of institution:	University of Montreal, Montreal, Canada.
Contract No.:	DA-49-193-MD-2039.
Supported by:	U.S. Army Medical Research and Development Command, Department of the Army, Washington 25, D.C.

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In further exploration of different calciphylactic syndromes, it was shown that a variety of agents such as distilled water, mechanical trauma (pinching the skin with a hemostat), formaldehyde, croton oil, or histamine liberators (e.g., compound 48/80, polymyxin) selectively inhibit skin calcification at the point where they are applied within the challenged area. On the other hand, we found that in rats, topical direct calcinosis (e.g., without pretreatment with DHT), normally induced in the subcutaneous connective tissue at the site of $KMnO_4$-injection, is prevented if the animals are maintained on a diet virtually deficient in both calcium and phosphate.

We investigated a calciphylactic syndrome characterized by heavy calcium deposits in the snout, paws and esophagus induced by certain agents. Moreover, a technique was developed for the study of mast cells.

Selective necrosis with calcification at the corticomedullary junction of the kidney in the rat was produced in experiments with hexadimethrine bromide, an agent in clinical use for the inactivation of heparin.

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CALCIPHYLAXIS IN WOUND HEALING

During the period covered by this report, we pursued our investigations on this recently developed biologic reaction form -- Calciphylaxis -- which was introduced in our previous report as an essentially defensive hypersensitivity reaction, lending itself particularly well as an experimental tool for the study of connective-tissue reactions and that particular group of the so-called non-allergenic hypersensitivity reactions which apparently are not accompanied by any obvious developments of blood-borne immune bodies. Our studies have since been described in several publications; financial assistance provided under contract No. DA-49-MD-2039 is acknowledged in 9 scientific publications, including a Ph.D. thesis by Dr. R. Veilleux (see list of publications and collection of available reprints). This work may be summarized as follows:

Calciphylaxis is a defensive reaction, which, depending upon circumstances, can either produce calcification by concentrating calcium salts in more or less circumscribed foci, or prevent calcinosis by "deviation" or dispersion of the metal throughout the body. The concentrating form of calciphylaxis often provokes inflammation and sclerosis through the selective deposition of irritating calcium salts in the challenged area; it can thereby help sequestrate a pathogen with granuloma tissue, thus increasing resistance to topical injury. However, this focal form of calciphylaxis can also become the cause of morbid lesions if an excessive amount of mineral is deposited in the tissues.

Deviating calciphylaxis, on the other hand, may be illustrated as follows: if a rat (weighing 100-200 g) is given 50 mg of iron intraperitoneally every five days in the form of a readily diffusible iron complex, such as ferric dextran

(Fe-Dex), it develops a generalized hemosiderosis owing to the formation of diffusely distributed, minute iron deposits. In animals thus pretreated, the most diverse forms of soft-tissue calcification are inhibited. Topical treatment with direct calcifiers no longer produces local calcification; heavy overdosage with dihydrotachysterol (DHT) or parathyroid hormone fails to elicit the customary calcification in the normally predisposed cardiovascular system, kidney or lung; and calciphylactic responsiveness to challengers is greatly diminished or totally suppressed. Apparently, here, the diffuse impregnation of the organism with a challenger results in protection against the induction of large focal calcium deposits. (see publications, Nos. 1 and 2).

However, large calcificed skin plaques can be produced if rats pretreated with DHT per os are calciphylactically challenged through the infiltration of a subcutaneous tissue area with dilute solutions of Fe-Dex. A variety of agents such as distilled water, mechanical trauma (pinching the skin with a hemostat), formaldehyde, croton oil, or histamine liberators (e.g., compound 48/80, polymyxin) selectively inhibit this skin calcification at the point where they are applied within the challenged area. Histologic studies show a relationship between this form of skin calcification, the distribution of mast cells and the deposition of iron. As a working hypothesis it is assumed that nonspecific topical stress can inhibit calciphylaxis even without producing evident signs of local injury, such as inflammatio or necrosis. This anticalciphylactic effect appears to be one of the most sensitive indicators of local stress; it is accompanied by changes in the mast cells and in the distribution of the challenger (here iron). It remains to be shown, however, whether these diverse manifestations of mild local stress are causally connected (see publication No. 3).

It has further been shown that in rats, topical direct calcinosis, normally induced in the subcutaneous connective tissue at the site of $KMnO_4$-injection, is prevented if the animals are maintained on a diet virtually deficient in both calcium and phosphate. Replacement with calcium acetate does not alter this refractory state, while the administration of Na_2HPO_4, either alone or in combination with calcium

acetate, restores normal reactivity. It is concluded that, for the production of topical calcinosis with $KMnO_4$, the dietary intake of phosphate is of decisive importance, while the calcium content of the diet plays little or no role in this phenomenon (see publication No.4).

Past experiments showed that, following administration of DHT, rats respond to the injection of certain metallic compounds by a typical syndrome characterized by heavy calcium deposits in the snout, paws and oesophagus. Recent findings indicate that it is the dextrin fraction of these compounds which causes such anaphylactoid phenomena prior to the calcifying response. It seems to us that this fact deserves special attention since dextrin is frequently used in the pharmaceutical compounding of certain drugs (see publication No. 5).

Since the mast cell is attracting increasingly more interest in connection with studies on release of heparin, histamine, serotonin, anaphylactic and anaphylactoid reactions, as well as calciphylaxis, we developed a technique for the study of mast cells on such "natural tissue spreads" as the external periosteum of the calvarium and the dura matter of small laboratory rodents. These membranes can be fixed as flat sheets, while still attached to their normal osseous base, without the use of the customary traumatic procedures incident to the preparation of artificial tissue mounts. (see publication No. 6).

Hexadimethrine bromide, an agent in clinical use for the inactivation of heparin, is claimed to cause disruption of tissue mast cells perhaps with a liberation of histamine, serotonin and heparin in the rat (Kimura et al., 1961;1962). Experiments conducted in our laboratories showed that hexadimethrine, given intravenously, can produce a selective necrosis with calcification at the corticomedullary junction of the kidney in the rat. This change is frequently accompanied by hemorrhages, adrenal necroses, periarteritis nodosa of the hepatic artery, osteitis fibrosa and a singular type of anaphylactoid reaction. It was also shown that the hexadimethrine-induced nephrocalcinosis can be inhibited, for instance, by oral administration of calcium acetate, or aggravated by similar treatment with sodium phosphate and various other agents. (see publication No. 7).

LIST OF PUBLICATIONS

1. - The dermatologic implications of stress and calciphylaxis. H. SELYE - J. Invest. Dermat., 39, 259 (1962). (4 reprints enclosed).

2. - Prevention by ferric dextran of the topical calcification induced by direct calcifiers. H. SELYE, B. TUCHWEBER, G. GABBIANI - Medicina Experimentalis, 7, 170 (1962). (4 reprints enclosed).

3. - Prevention of cutaneous calciphylaxis by topical stress. H. SELYE, B. TUCHWEBER and G. GABBIANI - Archives of Dermatology. (In press, reprints will follow when available).

4. - Prevention by dietary means of the direct calcification induced by $KMnO_4$. B. TUCHWEBER, G. GABBIANI and H. SELYE - Medicina Experimentalis. (In press, reprints will follow when available).

5. - Anaphylactoid oedema produced in rats by certain dextrin. R. VEILLEUX - Brit. Med. J. (In press, reprints will follow when available).

6. - The "periosteal spread" technique for the study of mast cell. H. SELYE, G. GABBIANI and K. NIELSEN - Proc. Soc. Exper. Biol. & Med., 112, 460 (1963). (4 reprints enclosed).

7. - Organ lesions produced by hexadimethrine and their modification by various agents. H. SELYE, G. GABBIANI and B. TUCHWEBER - Medicina Experimentalis. (In press, reprints will follow when available).

8. - Les myosites pluricausales expérimentales. J.M. DIEUDONNE and H. SELYE - Rev. canad. Biol., 21, 477 (1962). Internat. Sympos. Muscle Res., p. 477 (1962). (4 reprints enclosed).

9. - Inflammation, calciphylaxis and collagen disease. H. SELYE - Medicina Espagnola. Lecture, Instituto de Investigaciones Clinicas y Médicas, Madrid, Spain (1962). (In press, reprints will follow when available).